



PRODUCT SPECIFICATION SHEET

B12 Assay Agar (Using *E. coli* Mutant Culture) (Harrison et al. Medium) (DM528)

Intended Use

B12 Assay Agar (Using *E. coli* Mutant Culture) (Harrison et al. Medium) (DM528) is recommended for Vitamin B12 assay using *Escherichia coli* mutant 113-3 Davis ATCC 11105.

Product Summary and Explanation

B12 Assay Agar (Using *E. coli* Mutant Culture) is prepared as per the formula described by Harrison et al. B12 Assay Agar is a vitamin B12-free dehydrated medium containing all other nutrients and vitamins essential for the growth and cultivation of *E. coli* mutant 113-3 Davis ATCC-11105. Inclusion of Vitamin B12 in specified increasing amounts gives a growth response that can be measured by the diameter of the zone of growth around the disc or cup containing Vitamin B12.

Principles of the Procedure

B12 Assay Agar (Using *E. coli* Mutant Culture) (Harrison et al. Medium) contains all the nutrients required for the growth of *E. coli* mutant 113-3 Davis ATCC-11105 except vitamin B12.

Formula / Liter

For microbiological assay of Vitamin B12, a complete dehydrated medium contains all essential nutritives except Vitamin B12 for the growth of *E. coli* mutant 113-3 Davis ATCC11105. Addition of B12 in specified increasing concentration gives a growth response, which can be measured with zone reader.

Final pH : 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Over heating or over sterilization will give unsatisfactory results.

Directions

1. Suspend 51.5 grams of the medium in one litre distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Mix well to distribute slight precipitate evenly.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Generally satisfactory results are obtained with B12 at levels ranging from 0 to 300 ng per ml.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Medium amber clear to slightly opalescent gel forms in Petri plates
Reaction of 5.15% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Microbiological assay of Vitamin B12 was carried out using *E. coli* mutant 113-3 Davis ATCC 11105 as a test organism. Cultural characteristics observed after an incubation at 35- 37°C for 18-24 hours, good growth was obtained around cups containing Vitamin B12 showing an increase in diameter of zone of growth in proportion the increasing Vitamin B12 concentration in the cup.





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Test Procedure

Standard Preparation:

Prepare sterile solutions of Vitamin B12 (Cyanocobalamine Reference Standard).

Determination of Vitamin B12 content:

For determination of vitamin B12 content of unknown materials the assay sample should be properly diluted and applied similarly as the dilutions of the standards.

Inoculum Preparation:

Inoculum for the assay is prepared by sub-culturing from a stock culture previously made by stab inoculation. Freshly sub-cultured cells incubated at 35°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for this assay.

Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium below 10-30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : B12 Assay Agar (Using E. coli Mutant Culture) (Harrison et al. Medium)

Product Code : DM528

Available Pack sizes : 100gm

References

1. Harrison, E., Lees, K.A and Wood, F. (1951) Analyst 76: 696.

Further Information

For further information please contact your local MICROMASTER Representative.





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